Postharvest Berry Drop of Seedless Berries Produced by GA Treatment in Grape Cultivar 'Kyoho' IV. Rachis Hardness and Phenol Production of Rachis in Vitro Following GA Application as Affected by the Addition of β -Nitrostyrene Derivatives

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Postharvest Berry Drop of Seedless Berries Produced by GA Treatment in Grape Cultivar 'Kyoho'

IV. Rachis Hardness and Phenol Production of Rachis *in Vitro* Following GA Application as Affected by the Addition of β-Nitrostyrene Derivatives

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Summary

β-Nitrostyrene derivatives (hereafter abbreviated as NSDs for convenience) and phenyl nitro alkanols were surveyed to determine their efficacy in preventing GA-induced rachis hardening in 'Kyoho', when added at 1000 ppm to 100 ppm GA, and *trans*-1-(4,5-dimethoxyphenyl)-2-nitroethene-1(NSD-No. 9) was shown to be most effective based on its effect on the deflection angle of rachis, which was inversely related to the rachis hardness. Moreover, NSD-No. 9 added to GA decreased the number of cell layers of secondary xylem in transection by only 2 cells, compared with GA alone. Further, in the experiments with 500 ppm NSD-No. 9, its addition to 100 ppm GA increased the deflection angle of both rachis and tendril to above 40 % of that of untreated ones, while, that of rachis and tendril treated with GA alone was only about 13 to 25 %. The thickness of secondary xylem of rachis and pedicel was not little affected similarly in the number of its cell layers. It also had no adverse effects on the growth of clusters and berries, the number of berries per cluster, and percentage of seedlessness. The horizontal fruit removal force (FRF) was not increased by its addition, but kept at the same level as that when GA alone was used, in spite of a relatively high total soluble solids (TSS) content. Further, the phenol production by rachis segments in liquid culture per unit fresh weight and unit surface area greatly decreased with increasing concentrations of NSD-No. 9. From these results, NSD-No. 9 proved to be effective, though not sufficient for practical use, in preventing the GA-induced rachis hardening perhaps by reducing rachis lignification via its inhibitory effect on PAL activity.

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Previously we suggested that GA-induced rachis hardening was mainly due to the increase in the number of cell layers of secondary xylem of rachis in transection in 'Campbell Early', while due to the lignification of the secondary xylem cells, perhaps, via increased PAL activity in 'Kyoho', though only comparatively (1, 2). Recently, PAL activity was reported to be inhibited in potato tubers (3, 4, 5, 6) and other plants (7, 8, 9) by exogenous applications of some phenolic acids as precursors in lignin biosynthesis. NSDs and phenyl nitro alkanols having chemical structures similar to phenolic acids were developed as microbicides or insecticides (10, 11, 12), and suspected to have some inhibitory effect on lignification (private communication from Dr. Koremura).

The objective of this study was to investigate the effects of NSDs and phenyl nitro alkanols added to GA on the prevention of GA-induced rachis hardening based on the assumption that the control of PAL activity might be effective in preventing rachis hardening especially in 'Kyoho'.

Materials and Methods

Throughout this study, 6-year-old 'Kyoho' and GA_3 as gibberellin (GA) were used.

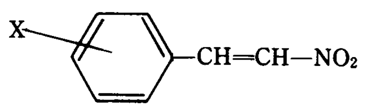
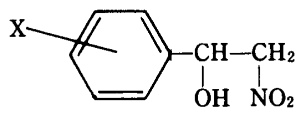
Experiment I

Clusters were dipped into the aqueous solutions of 100 ppm GA plus each of 16 kinds of NSDs and 3 kinds of phenyl nitro alkanols at 1000 ppm. As controls, clusters dipped into the solution of GA alone and others not dipped at all were prepared. All solutions contained 100 ppm Aerol OP as a wetting agent throughout this study. Treatments were carried out 6-7 days after full bloom in 4 replications. The chemical structures of the reagents tested are shown in Table 1. The clusters were trimmed before berry set, but not thinned thereafter. They were harvested on September 29 and the cluster and berry weight, deflection angle of rachis, diameter of rachis and width of its xylem at the fulcrum were measured as reported previously (13). The deflection angle of treated rachises was expressed in percentages of those left untreated. After the measurements, the rachises were fixed in 50 % alcohol and the number of cell layers of secondary xylem in rachis transection was counted in 5 replications as described previously (1).

Experiment II

The second and third tendrils from the shoot tip were dipped into the aqueous solutions of 100 ppm GA plus 500 ppm (instead of 1000 ppm in Expt. I) NSD-No. 4, 9, 12, 14 or 16 in 5 replications on May 30. These 5 NSDs had been shown to be effective in Expt. I. As controls, tendrils dipped into the solution of GA alone and others not dipped at all were prepared. The tendrils were harvested on June 30. The deflection angle of tendril at 2 cm proximal portion from the first branch

TABLE 1. *Formulas of β-nitrostyrene derivatives (NSDs) and phenyl nitro alkanols*

β-Nitrostyrene derivatives			Phenyl nitro alkanols		
					
No.	X		No.	X	
1	H	(*p*)	17	H	(*p*)
2	Cl	(*p*)	18	Cl	(*p*)
3	HO_2	(*p*)	19	$(CH_3O)_2$	(4, 5)
4	CH_3	(*p*)			
5	HO	(*p*)			
6	CH_3O	(*p*)			
7	$CH_3O(OH)$	(3, 4)			
8	$CH_3O(OH)$	(4, 6)			
9	$(CH_3O)_2$	(4, 5)			
10	$(C_2H_5O)_2$	(4, 5)			
11	$CH_2<\genfrac{}{}{0pt}{}{O}{O}$	(3, 4)			
12	NH_2	(*p*)			
13	CH_3-COHN	(*p*)			
14	$(CH_3)_2$-N	(*p*)			
15	Cl_2	(4, 6)			
16	NH_2SO	(*p*)			

of the tendril was measured.

Experiment III

Clusters were dipped into the aqueous solutions of 100 ppm GA plus 500 ppm NSD-No. 4, 9 or 14 at full bloom in 5 replications on June 18. As controls, clusters dipped into the aqueous solution of GA alone and others not dipped were prepared. The clusters were not trimmed nor thinned, and were harvested on August 8. The cluster weight, deflection angle of rachis, diameter of rachis and width of its xylem at the fulcrum were measured as described previously (13). The horizontal FRF, average berry weight, number of berries per cluster, TSS content, and diameter of pedicel and width of its xylem at berry attachment were measured separately for seeded and seedless berries, because both kinds of berries were found in a cluster regardless of treatments.

Experiment IV

The lowest clusters on the shoots were removed on July 15. After sterilized with 0.001 % benzalkonium chloride for 2 h, the third internode from the shoulder of rachis was dissected into 1-cm segments. The segments were resterilized with 70 % ethanol and then with filtrate of 10 % chlorinated lime for 10 min. After rinsed in sterilized water, one segment per vial was put into 20 ml of MS liquid medium and cultured as described previously (2). GA and/or NSD-No. 9 were added to culture medium through millipore filters with 0.45-μm pore size at the final concentrations of 100 ppm and 10.0, 1.0 and 0.1 ppm, respectively. After 7 days of liquid culture, the fresh weight, diameter and length of segments and the total phenols dissolved into the medium were determined in 6 replications as described previously (2).

Results

1. Deflection Angle of Rachis and Number of Cell Layers of Secondary Xylem of Rachis as Affected by NSDs and Phenyl Nitro Alkanols Added to GA (Experiment I).

Among the reagents tested, NSD-Nos. 4, 9, 12, 14 and 16 added at 1000 ppm to 100 ppm GA more or less increased the deflection angle of rachis as compared with GA alone. NSD-No. 9 was especially effective and the deflection angle was 2.2 times that of rachis treated with GA alone (Fig. 1). The thickness of rachis and its xylem treated with GA plus the 5 NSDs inclusive of No. 9 differed little from each other and, moreover, that of the rachis xylem differed little from that of rachis treated with GA alone (Fig. 2). In 'Campbell Early', however, NSD-No. 12 was most effective in increasing the deflection angle of rachis (data not shown), but its effect was not further investigated.

The number of cell layers of secondary xylem in transection of rachis treated with GA plus NSD-No. 9 was only 2 cells less than that of rachis treated with GA

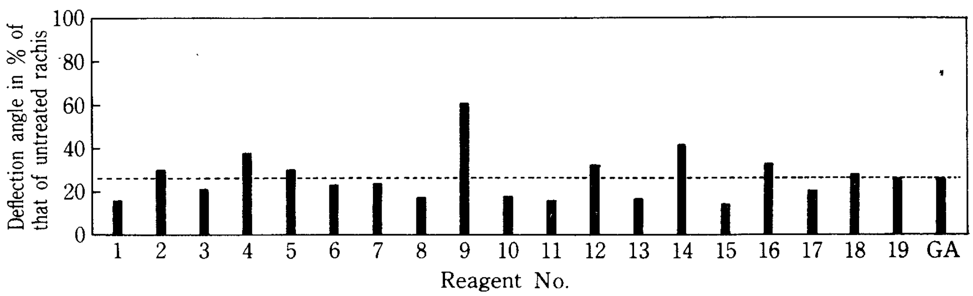


FIG. 1. Effects of 1000 ppm NSDs and phenyl nitro alkanols added to 100 ppm GA on deflection angle of rachis when the weight of 50 g is hung at a distance of 10 cm from the fulcrum in 'Kyoho'. Horizontal dotted line indicates the value of rachis treated with GA alone.

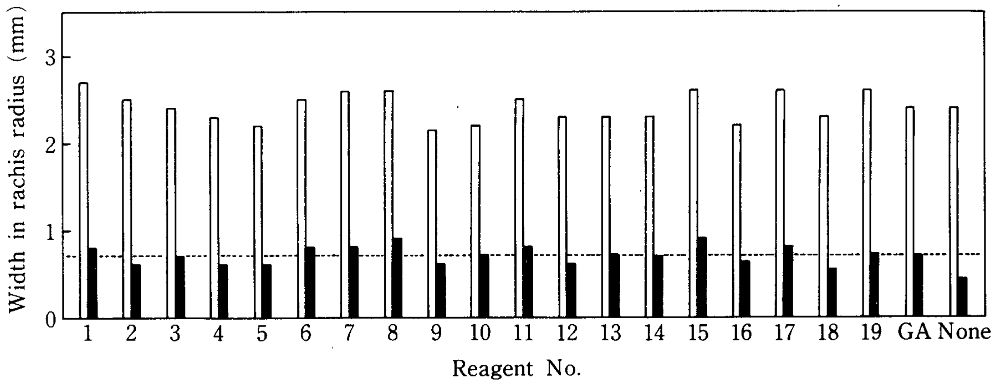


FIG. 2. Effects of 1000 ppm NSDs and phenyl nitro alkanols added to 100 ppm GA on radial diameter of rachis and width of secondary xylem layer in 'Kyoho'. Rachis : □. Rachis xylem : ■. Horizontal dotted line indicates the value of rachis xylem treated with GA alone.

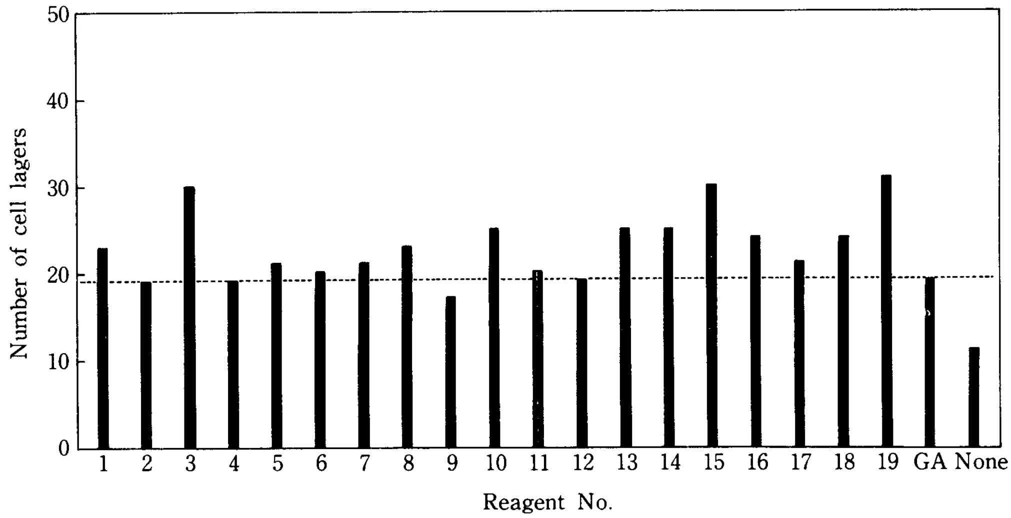


FIG. 3. Effects of 1000 ppm NSDs and phenyl nitro alkanols added to 100 ppm GA on the number of cell layers of secondary xylem in rachis transection in 'Kyoho'. Horizontal dotted line indicates the value of rachis treated with GA alone.

alone. While, that of rachis treated with GA plus NSD-No. 4 or 12 was almost the same with, and that of rachis treated with NSD-No. 14 or 16 was 5-6 cells more than that of rachis treated with GA alone (Fig. 3).

2. Deflection Angle of Tendril and Rachis and Cluster and Berry Growth as Affected by NSD-Nos. 4, 12, 14 and 16 and Especially of No. 9 Added to GA (Experiment II and III).

The deflection angle of the second and third tendrils from the shoot tip treated with 100 ppm GA alone was only 24.5 and 13 %, respectively, of that of untreated ones, but they increased to 42 and 44 % by the addition of 500 ppm NSD-No. 9, while, the addition of NSD-Nos. 4, 12, 14 and 16 had little effect (Fig.

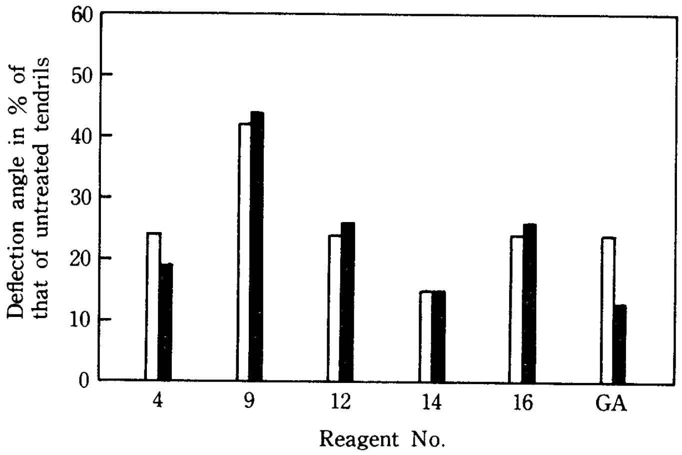


FIG. 4. Effect of 500 ppm NSDs (Nos. 4, 9, 12, 14 and 16) added to 100 ppm GA on deflection angle of the second and third tendrils from the shoot tip when the weight of 50 g is hung at a distance of 10 cm from the fulcrum. The second tendril : ■. The third tendril : □.

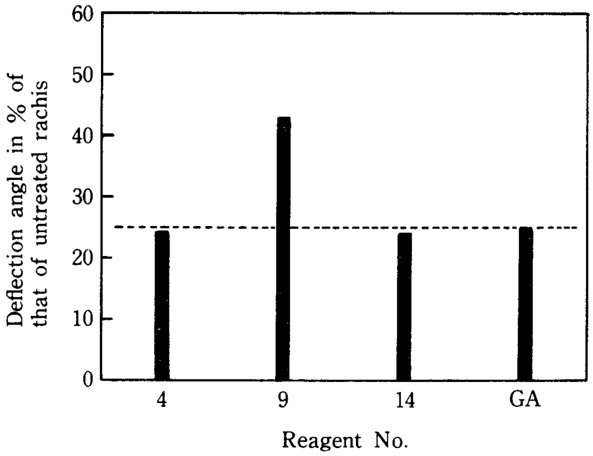


FIG. 5. Effect of 500 ppm NSDs (Nos. 4, 9 and 14) added to 100 ppm GA on deflection angle of rachis when the weight of 50 g is hung at a distance of 10 cm from the fulcrum. Horizontal dotted line indicates the value of rachis treated with GA alone.

4). Also, the deflection angle of rachis treated with GA alone was only 25, while it was increased to 43 by the addition of NSD-No. 9 in percentage, compared with untreated ones. NSD-No. 4 and 14 had little effect as in the case of tendrils (Fig. 5). The thickness of secondary xylem of rachis and pedicel treated with GA plus NSD-No. 9 was almost the same as that of rachis and pedicel treated with GA alone (Table 2). NSD-No. 9 had no adverse effects on cluster weight, seedless berry weight, number of berries per cluster and percentage of seedlessness. The horizontal FRF was also little affected, although TSS content of berries was slightly increased by the addition of NSD-No. 9 (Table 3).

TABLE 2. *Effect of 500 ppm NSDs (Nos. 4, 9 and 14) added to 100 ppm GA on widths of rachis and pedicel and their secondary xylem layers*

	Rachis		Pedicel	
	Width (mm)	Secondary xylem width (mm)	Width (mm)	Secondary xylem width (mm)
GA 100 ppm+NSD-No. 4 500 ppm	4.8	0.6	4.1	0.8
GA 100 ppm+NSD-No. 9 500 ppm	4.5	0.5	3.9	0.8
GA 100 ppm+NSD-No. 14 500 ppm	4.6	0.7	3.9	0.8
GA 100 ppm	4.1	0.5	4.2	0.8
Untreated	3.8	0.3	4.5	0.7

TABLE 3. *Effect of 500 ppm NSDs (Nos. 4, 9 and 14) added to 100 ppm GA on cluster weight, seedless berry weight, number of berries per claster, fruit removal force (FRF) and total soluble solids (TSS) content*

	Cluster wt (g)	Seedless berry wt (g)	No. of berries per cluster		FRF (g)	TSS (%)
			Seeded	Seedless		
GA 100 ppm+NSD-No. 4 500 ppm	283	7.3	3	40	106	12.8
GA 100 ppm+NSD-No. 9 500 ppm	237	6.9	3	34	105	12.2
GA 100 ppm+NSD-No. 14 500 ppm	198	6.2	4	31	101	12.1
GA 100 ppm	190	7.8	7	21	104	11.3
Untreated	118	7.4	16	2	176	9.4

TABLE 4. *GA-induced phenol production by rachis segments in liquid culture as affected by addition of NSD-No. 9 at different concentrations*

	Total phenols produced* (mg)	Fresh wt/ segment (g)	Total phenols produced/ fresh wt (mg/g)	Surface area/ segment (cm^2)	Total phenols produced/ surface area (mg/cm^2)	Segment width (cm)
Untreated	0.13	0.42	0.32	1.90	0.07	0.48
GA 100 ppm	0.21	0.36	0.57	1.77	0.12	0.45
GA 100 ppm +NSD-No. 9 0.1 ppm	0.20	0.48	0.42	2.06	0.10	0.50
GA 100 ppm +NSD-No. 9 1.0 ppm	0.13	0.39	0.33	1.77	0.07	0.44
GA 100 ppm +NSD-No. 9 10.0 ppm	0.03	0.95	0.03	1.99	0.01	0.49

* mg gallic acid equivalent per culture (20 ml).

4. Inhibition of GA-induced Phenol Production by Addition of NSD-No. 9 in Rachis Segments in Liquid Culture (Experiment IV).

The total phenols dissolved into the liquid medium per unit fresh weight (mg/g) and per unit surface area (mg/cm^2) were only 0.32 and 0.07, but they were increased to 0.57 and 0.12, respectively, by the addition of 100 ppm GA. As the concentration of NSD-No. 9 added to 100 ppm GA was increased from 0.1 to 10.0 ppm, however, they decreased from 0.42 to 0.03 and from 0.10 to 0.01, respectively (Table 4).

Discussion

NSDs and phenyl nitro alkanols were shown to be effective as microbicides or insecticides (10, 11, 12) and were suggested to have some inhibitory effect on lignification due to a kind of inhibition by product analogues (private communication from Dr. Koremura). Among them, NSD-Nos. 4, 9, 12, 14 and 16, and particularly NSD-No. 9, were shown to be effective in increasing the deflection angle of rachis when added at 1000 ppm to 100 ppm GA. The deflection angle of rachis was already shown to be inversely related to the rachis hardness associated with the easiness of berry drop (13).

With the NSDs more or less effective and, in particular, NSD-No. 9, their effects on the hardening of rachis together with the tendril having a common origin with the cluster, and on the growth of cluster and berries were further investigated. Again, NSD-No. 9 decreased most effectively the rachis hardness though not enough for practical use. In both the rachis and tendril, the deflection angle increased to above 40 % of that of untreated ones by its addition to GA, as compared with about 25 % when treated with GA alone. NSD-No. 9 had little or no effect on the thickness of secondary xylem of rachis and pedicel, suggesting little effect on the number of the secondary xylem cells, which had already been shown in Experiment I. NSD-No. 9 had no adverse effects on the growth of cluster and berries nor GA-induced seedlessness. Contrary to our expectations, we found no increase in the horizontal FRF, which seemed, in part, to be due to the advanced maturity inferred from the small increase in TSS content. Moreover, the total phenol production by the rachis segments in liquid culture decreased markedly when NSD-No. 9 was added to the medium together with GA.

Thus, in 'Kyoho', among the reagents tested, NSD-No. 9 proved to be the most effective in preventing GA-induced rachis hardening when added to GA, mainly owing to the reduction of lignification of secondary xylem cells of rachis. Some phenolic acids, mainly *trans*-cinnamic acid, have been reported to inhibit PAL activity in tobacco (7), potato tubers (3, 4, 5, 6), beans (8) and gherkin (9). NSD-No. 9 seemed to prevent lignin production perhaps by inhibiting PAL activity as analogues and at the same time being ineffective as the precursor in lignin biosynthesis in itself.

Although NSD-No. 9 is not sufficiently effective for practical use, its effects do suggest the possibility of obtaining more useful chemicals to prevent rachis hardening. In addition, in 'Campbell Early', NSD-No. 12, instead of No. 9, was shown to most effectively increase the deflection angle of rachis. We hope for further investigation to reveal whether its effect is associated with the number of secondary xylem cells of rachis or their lignification.

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